THE ROUTINE CULTURAL IDENTIFICATION OF MICROSPORUM RINGWORM OF THE SCALP*

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Tinea capitis in the United States is caused principally by Microsporum audouini and M. canis (lanosum). Identification of the responsible fungus is important in determining the prognosis. Identification of M. audouini by the usual method of planting infected hairs on agar media in a test tube requires 7 to 14 days. M. canis colonies are recognizable in 5 to 8 days.

The method described below permits reduction in the time required for identification. It is recommended only for routine separation of M. canis, M. audouini and M. fulvum. We have not applied it in the differentiation of scalp ringworm due to species of Trichophyton. These latter organisms are distinctly uncommon as a cause of tinea capitis in the northeastern United States.

Hairs infected with Microsporum species exhibit a characteristic bright blue-green fluorescence. Hairs infected with the various species of Trichophyton either do not fluoresce, or exhibit a light bluish fluorescence which is readily distinguishable from that caused by Microsporum species.

Method: The hair to be cultured is first viewed under the Wood's light. This permits immediate recognition of the Microsporum infected hair. If any doubt remains concerning the fluorescence, an hydroxide preparation will generally confirm the presence of the small-spored ectothrix Microsporum.

The hairs are planted directly into a drop of liquid medium on a glass slide. Honey media or other Saboraud-like media are satisfactory. The routine medium used in this study was as follows:

Honey	60 gm.
Neopeptone (Difco)	10 gm.
Agar	1.5 gm.
Water	1 liter

The medium is made up in 250 ml. flasks, to which streptomycin and penicillin are added in a concentration of 50 units of each antibiotic per ml.

The glass slide is mounted on a U-shaped piece of glass tubing in a petri dish. Two pieces of 19 cm. filter paper are placed in the bottom of the dish (Fig. 1). The dish and its contents are sterilized. Just before use, enough sterile water to wet the filter paper liberally is poured from a flask into the petri dish, which thus serves as a moist chamber. Two moderately large drops of culture medium are placed an inch apart on the slide with a sterile pipette. The agar in the medium prevents the drops from spreading over the slide. One or two infected hairs are placed in each drop and the preparation is incubated at room temperature.

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The ectothrix spores of M. canis regularly germinate in one day. In 2 to 3 days the brilliant yellow pigment characteristic of the species develops in the medium around the hair shaft. In 3 to 5 days from the time of planting, the characteristic macroconidia are seen abundantly under low power on the aerial hyphae above the hair. The macroconidia develop with extraordinary speed. A 3 day culture which shows no macroconidia may have enormous numbers of mature macroconidia 12 hours later. Numerous macroconidia may be observed after the 5th day of incubation.

Germination of the slower growing M. audouini occurs in one to two days. In five to six days the colony is white to cream colored. Macroconidia are not regularly produced and hence cannot be used for identification. The identification depends on exclusion and is made after microscopic examination fails to reveal the presence of a contaminant or of macroconidia characteristic of M. canis. After a little experience one can recognize M. audouini grossly, although we

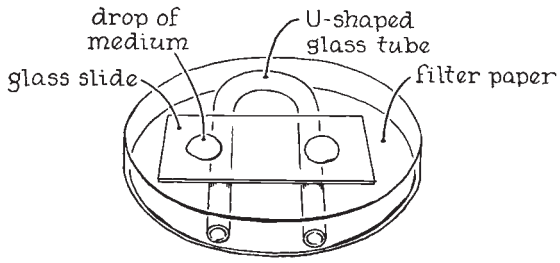

FIG. 1

routinely examine all cultures microscopically. Terminal or sub-terminal chlamydospores are frequently observed in M. audouini but not with sufficient regularity to be of diagnostic value. If there is any doubt as to whether the culture is a dermatophyte or not it may be held for eight or nine days, at which time numerous macroconidia appear.

The rapid growth in these slide cultures doubtless results from the fact that the fungi grow in a water saturated atmosphere. If the medium is permitted to spread in a film on the slide, the yellow color of M. canis will not be conspicuous.

To check the method, infected hairs from 132 consecutive cases of tinea capitis due to Microsporum were cultured. Hairs were planted on a slide and also on a potato dextrose agar (Difco) and a Saboraud dextrose agar (Difco) slant.

There were 11 cases of M. canis infection in the series, and identification was possible in every instance on both the slides and agar slants. M. audouini was identified 116 times on the slides and 111 times on the slants. In two instances on the slides, fungus contamination made identification impossible. One of these was recognized as M. audouini on the slants. (In three instances there was no growth on either slides or slants.) Thus, there were six instances in which identification failed on the slants and was possible on the slides. On the other hand, the slide method failed in one instance in which the agar slant was successful. The

addition of penicillin and streptomycin to the agar media would probably have reduced the failure rate of the slants.

Microsporum fulvum has not been encountered in this series. However, this species grows rapidly when inoculated on the slide and develops typical macroconidia in three to four days.

Thus, M. canis infected hairs may be identified presumptively in 2 to 3 days, and certainly in 3 to 5 days when macroconidia have developed. M. audouini infected hairs are identified in 5 to 6 days by noting the absence of yellow pigment, the lack of macroconidia, the lack of spore forms indicative of a contaminant, and the presence of a white to cream colored colony consistent with the appearance of M. audouini in slide culture.

Zimerinov and Rafalovich have planted hairs naturally infected with species of Trichophyton in hanging drops of beer-wort medium (1). They find T. violaceum, T. schoenleini, T. mentagrophytes, and T. tonsurans separable within one week. Because of the number of Trichophyton species which infect the hair, not all of which produce dependable microscopic structures, the method described in this paper is suggested only for Microsporum ringworm. The use of the slide culture and the hanging drop for the culturing of fungus infected tissue is not new and has been employed by others (2, 3). It has not, however, been employed in the routine identification of Microsporum species.

SUMMARY

A slide culture method of identifying Microsporum infected hairs in about six days is described. In a series of 132 cases the method proved at least as reliable as the conventional method of planting hairs on agar slants.

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